

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	10.09.97	Final report 11.01.93-10.31.96
4. TITLE AND SUBTITLE The role of interferon in the cellular response of the CNS macrophage, the microglia, during injury and inflammation.		5. FUNDING NUMBERS N00014-91-J-1123
6. AUTHOR(S) Carol A. Colton, Ph.D.		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Georgetown University Medical School 3900 Reservoir Rd. NW Washington, DC 20007		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research 800 N. Quincy St. Arlington, VA 22217-5000		10. SPONSORING/MONITORING AGENCY REPORT NUMBER
11. SUPPLEMENTARY STATEMENT DISTRIBUTION STATEMENT IS Approved for public release; Distribution Unlimited		
12a. DISTRIBUTION / AVAILABILITY STATEMENT Distribution unlimited		
13. ABSTRACT (Maximum 200 words) Our lab has studied the response of the CNS macrophage, the microglia to injury and inflammation. Using an <i>in vitro</i> approach, we have shown that microglia cultured from the cerebral cortices of neonatal animals (rat, mouse, hamster or human) have the same functional responses as non-CNS macrophages. That is, they demonstrate chemotaxis, express macrophage-like surface antigens and they produce a variety of cytoactive factors including proteases, interleukin-1 and reactive oxygen species (superoxide anion and nitric oxide). We found that both inflammatory and immune mediators (lipopolysaccharide and interferons, respectively) enhance the production of superoxide anion but do not directly activate the NADPH oxidase. These agents also increase nitric oxide (NO) production but in a very different time frame than that found for superoxide anion. Treatment of microglia with isoproterenol or dexamethazone depressed the microglial production of ROS. Our studies also demonstrated that human and hamster microglia do not produce NO in response to the same stimulating factors used in rat or mouse microglia. Hamster and human microglia did not produce NO except when treated with the double stranded polyribonucleotide, poly inosinic acid: poly cytidylic acid (Poly I:C). These findings have important consequences to the understanding of the response of humans to inflammation or injury.		

14. SUBJECT TERMS microglia, interferon, inflammation, injury	15. NUMBER OF PAGES 4		
DATA QUALITY INFORMATION			
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT UL

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to *stay within the lines* to meet *optical scanning requirements*.

Block 1. Agency Use Only (Leave blank).

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C - Contract	PR - Project
G - Grant	TA - Task
PE - Program Element	WU - Work Unit
	Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. (If known)

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement.

Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."

DOE - See authorities.

NASA - See Handbook NHB 2200.2.

NTIS - Leave blank.

Block 12b. Distribution Code.

DOD - Leave blank.

DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.

NASA - Leave blank.

NTIS - Leave blank.

Block 13. Abstract. Include a brief (*Maximum 200 words*) factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code (*NTIS only*).

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FINAL REPORT

GRANT #: N00014-91-J-1123 R&T CODE 4414138

PRINCIPAL INVESTIGATOR: Carol A. Colton, Ph.D.

INSTITUTION: Georgetown University Medical School

GRANT TITLE: The role of interferon in the cellular response of the CNS macrophage, the microglia, during injury and inflammation.

REPORTING PERIOD: 1 November 1993 to 31 October 1996

AWARD PERIOD: 1 November 1993 to 31 October 1996

OBJECTIVE: To investigate the response of the CNS macrophage, the microglia, to factors involved in the response to injury and infection. To investigate the role of interferon in this response and to determine potential sources of alpha/beta and gamma interferon in the CNS.

APPROACH: Primary microglia and astrocyte cultures are prepared from neonatal mouse or hamster cerebral cortices. At 14 days, microglia are separated from the underlying astrocyte layers by shaking and both cell populations, i.e., the microglia and the remaining astrocytes, are used in the experimental protocols. In some cases, primary cultures of human fetal astrocytes or human adult microglia were used in collaboration with Dr. M. DuBois-Dalg and Dr. S. Wilt, NIH.

ACCOMPLISHMENTS: Over the duration of this contract our lab has studied the response of the CNS macrophage, the microglia to injury and inflammation. Although microglia are of monocytic origin, it was unclear at the onset of the project if these cells responded in a similar fashion as other tissue macrophages. Using an *in vitro* approach, we have shown that microglia cultured from the cerebral cortices of neonatal animals (rat, mouse, hamster or human) have the same functional responses as non-CNS macrophages. That is, they demonstrated chemotaxis, express macrophage-like surface antigens which may be used to identify the cell in the CNS, and they produce a variety of cytoactive factors including proteases, interleukin-1 and notably, reactive oxygen species (superoxide anion and nitric oxide). Because these cells are found in all regions of the brain, including the hypothalamic-pituitary axis, it was of interest to examine the ability of neuroendocrine factors to modulate the activity of microglia. Such modulation serves as a bridge between the neuronal and immune systems. We found that both inflammatory and immune mediators (lipopolysaccharide and interferons, respectively) enhance the production of superoxide anion but do not directly activate the NADPH oxidase. These agents also increase nitric oxide (NO) production but in a very different time frame than that found for superoxide anion production. This difference has important consequences to the potential production (or lack of production) of peroxynitrite, a putative powerful oxidant involved in oxidative damage of cells. The stress-related hormones, namely isoproterenol, dexamethasone, corticotropin releasing hormone (CRH) and adrenocorticotropin (ACTH) also affect microglia. Our studies show that pretreatment for either 30 minutes or 24 hours with high doses of isoproterenol decreased phorbol myristate acetate (PMA)-stimulated superoxide anion production. This effect was reversed with, propranolol, a known β -adrenergic receptor blocking agent. Forskolin, an agent known to increase cAMP levels by direct activation of adenylyl cyclase, also depressed PMA-stimulated superoxide anion production but only when the microglia were exposed to

forskolin for short durations. Longer exposure (i.e., 24 hour pre-treatment) had no effect on PMA-stimulated superoxide anion production. Immunoreactivity for *c-fos* and *c-jun*, products of the early response genes, was increased in both forskolin and isoproterenol pre-treated microglia.

The action of dexamethasone on PMA-stimulated superoxide anion production was also studied. Dexamethasone significantly decreased superoxide anion production and this effect was reversed by the addition of cyclohexamide, indicating that protein synthesis was essential to the inhibitory effect of dexamethasone.

A major component of these studies has been examination of the species differences in NO production. Our studies were the first to demonstrate that human and hamster microglia do not produce NO in response to the same stimulating factors used in rat or mouse microglia (1). All microglia were stimulated with lipopolysaccharide (LPS), interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α) and combinations of these factors for 1 to 5 days. The level of nitrite in the supernatants was then compared to untreated controls. For hamster microglia, no detectable nitrite level above background was found for any of the stimulation conditions or at any of the longer durations of treatment. Likewise, human microglia did not demonstrate a significant increase in nitrite levels above untreated for any stimulation conditions studied or for any duration of treatment. Human fetal astrocytes did demonstrate a significant increase in nitrite levels in cells treated with the combination of TNF- α , IL-1 β and LPS compared to untreated. Our recent studies have further demonstrated that viral mediators can induce NO production (2). Treatment of hamster microglia or human monocyte derived macrophages (MDM) with the double stranded polyribonucleotide, poly inosinic acid: poly cytidylic acid (Poly I:C) induced measurable nitrite formation which was inhibitable by N-monomethyl arginine (NMMA), a known inhibitor of nitric oxide synthase (NOS). This effect was also seen if poly I was used but not poly C and was primed by gamma or alpha interferon or other immune mediators such as interleukin 4.

In collaboration with Dr. A. Namboodiri, Department of Biology, Georgetown University, we have studied the production of quinolinic acid (QUIN) in both hamster and human microglia. The immunostaining studies indicate that QUIN is present in adult human microglia and not in human astrocytes. This level does not, however, change with stimulation using LPS or γ IFN. This increase in measurable levels coincided with the presence of QUIN immunoreactivity in the cells. The level of QUIN in MDM, however, was significantly greater than that found in microglia under the same treatment conditions. Stimulated QUIN production by cultured astrocytes was not significantly increased over resting levels.

For hamster microglia, specific QUIN staining was not detectable because of a high background signal of unknown nature.

SIGNIFICANCE: Our data demonstrate that microglia function as a CNS macrophage and, as such, are part of the immune system of the CNS. The microglia are responsive to typical immune and inflammatory mediators and like other tissue macrophages, secrete a variety of cytoactive factors which kill invading organisms but also orchestrate the repair of the tissue. Because neurons are post mitotic, however, macrophage activation in the CNS is associated with neuronal death. Stress-related neuroendocrines, such as nor-epinephrine (or the closely related analog, isoproterenol) and dexamethasone inhibit microglial superoxide anion production. If a similar phenomenon occurs in vivo, the inhibition of

immune effector cell function could have important consequences to the CNS and to the immune response in the CNS. This mechanism may, however, serve a protective function potentially reducing neuronal death associated with microglial activation.

Our recent studies on human and hamster microglia further support the idea that major species differences exist in the regulation of microglia and astrocytes. Of the species studied, only rat and mouse microglia and astrocytes produce significant quantities of NO while human and hamster microglia and astrocytes do not apparently generate NO. The term "low output" NO has been applied to human macrophages while rat and mouse macrophages have been termed a "high-output" system. Only certain viral mediators activate NO production in human while mouse and rat cells respond to a variety of mediators. The dramatically different response patterns strongly suggests that caution must be used in correlating rat or mouse NO studies with human and treatment protocols based on manipulation of NO-mediated pathways.

PATENT INFORMATION: None

AWARD INFORMATION: Promoted to Full Professor

PUBLICATIONS AND ABSTRACTS:

Colton, C., Snell, J., Chernyshev, O. and D. Gilbert, Induction of superoxide anion and nitric oxide production in cultured microglia, Ann. N. Y. Acad. Sci., 738:54-63, 1994.

Colton, C., Pagan, F., Snell, J., Colton, J., Cummins, A. and D. Gilbert, Protection from oxidation enhances the survival of cultured mesencephalic neurons. Exp. Neurol., 132, 54-61, 1995.

Streit, W. and C. Kincaid-Colton, Microglia, Scientific American, 273, 38-43, 1995.

Colton, C. and O. Chernyshev, Inhibition of microglial superoxide anion production by isoproterenol and dexamethasone, Neurochemistry International, 29:43-53, 1996.

Colton, C., O. Chernyshev, J. Snell and D. Gilbert. Species differences in microglial responses. Society for Neuroscience Abstracts, 1994.

Colton, C., Induction of nitric oxide in cultured microglia: Evidence for a cytoprotective role, Adv. Neuroimmunol. 5, 491-503, 1995.

Espey, M., Chernyshev, O., Reinhard, J., Namboodiri, M. and Colton, C. Activated human microglia produce the excitotoxin quinolinic acid, NeuroReport, 8:431-434, 1997.

Snell, J., Chernyshev, O., Gilbert, D. and Colton, C., The effect of polyribonucleotides on nitric oxide production in hamster microglia and human macrophages, Neurosci. Abstract, 1996

Colton, C., Wilt, S., Gilbert, D., Chernyshev, O., Snell, J. and Dubois-Dalcq, M., Species differences in the generation of reactive oxygen species by microglia. Chem. Mol. Neuropathol., 28: 15-20, 1996.

Snell, J., Colton, C., Chernyshev, O. and Gilbert, D., Location dependent artifact for NO measurement using multiwell plates. Free Radical Biology and Medicine, 20, 361-363, 1996.

Espey, M., Reinhard, J., Chernyshev, O., Colton, C. and Namboodiri, M., Determination of quinolinic acid synthesis in human astrocytes and microglia in vitro, Neuroscience Abst. 1996.

Snell, J.C., Chernyshev, O., Gilbert, D. and **C.A. Colton**,
Polyribonucleotides induce nitric oxide production by human
monocyte-derived macrophages. *J. Leuko. Biol.*, 62, 369-373, 1997.

Vitek, M.P., Snell, J., Dawson, H. and **C. A. Colton**, Modulation of
nitric oxide production in human macrophages by apolipoprotein and
amyloid beta peptide., *Biochem. Biophys. Res. Comm.*, in press,
1997